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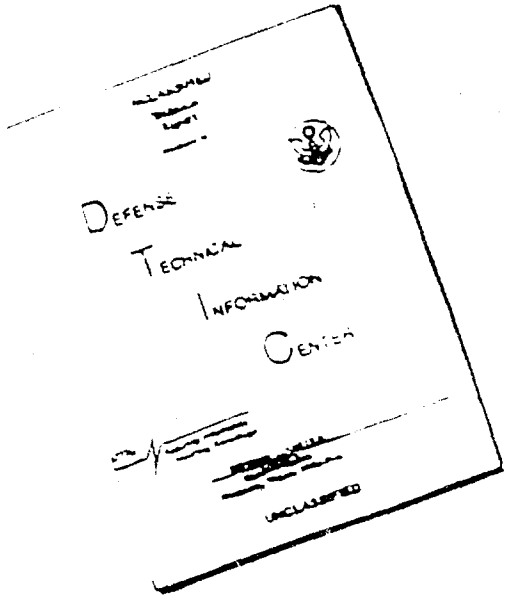

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EVALUATION OF 90-DAY INHALATION TOXICITY OF PETROLEUM AND OIL SHALE DIESEL FUEL MARINE (DFM)

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DECEMBER 1985

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Subchronic 90 day inhalation toxicity studies were conducted to compare the toxicity of Petroleum and Oil Shale Derived Diesel Fuel Marine (DFM). Beagle dogs, Fischer 344 rats, and C57BL/6 mice were continuously exposed to DFM at concentrations of 50 mg/m3 and 300 mg/m3. Unexposed controls were also maintained. All dogs and a portion of each rodent group were sacrificed and examined at exposure termination. The remaining rodents were held for observation up to 21 months postexposure. Male rats exposed to DFM for 90 days developed nephrotoxic changes characterized by hyaline degeneration, necrosis, and intratubular cysts. Subsequently, male rats exposed to 300 mg/m3 DFM developed mineralization and papillary hyperplasia. These postexposure renal changes were generally less severe in male rats exposed to 50 mg/m3 Shale DFM and were absent in male rats exposed to 50 mg/m3 Petroleum DFM. Female rats as well as dogs and mice exposed to DFM were free of significant renal damage. Mild reductions in body weight gains and erythrocyte parameters were also noted in male rats exposed to DFM.

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18. Nephrotoxicity
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Oil Shale Fuels

19. Neither material produced significant tumor formation in any species tested. The results of this study are consistent with the effects noted in other hydrocarbon fuel toxicity studies. Comparison of the effects observed in these studies with petroleum or shale suggest only minor differences between the two materials.

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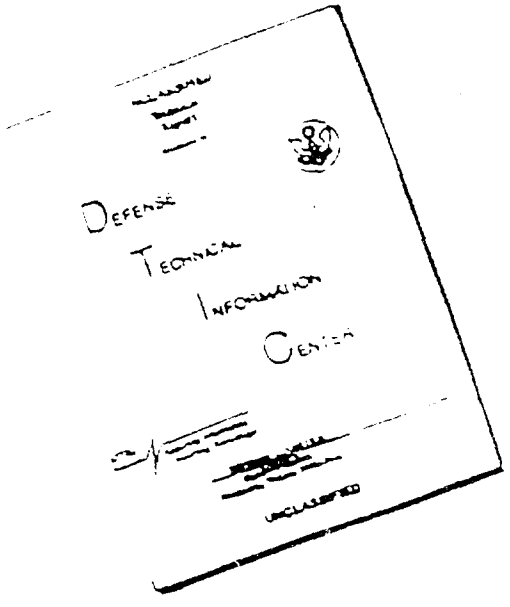

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PREFACE

This document constitutes the final report on the Evaluation of the 90-Day Inhalation Toxicity of Petroleum and Oil Shale Diesel Fuel Marine (DFM). The research covered a period from November 1977 through January 1985 and was performed under Contract No. F33615-80-C-0512. M. K. Pinkerton and K. C. Back, Ph.D. served as technical contract monitors for the Air Force Aerospace Medical Research Laboratory.

This work was sponsored by the U. S. Navy under the direction of CDR L. J. Jenkins, MSC, USN, Ret.; LCDR M. J. Cowan, Jr., MSC, USN, Ret.; LCDR L. L. Pitts, II, MSC, USN; and Capt. D. E. Uddin, MSC, USN; and identified as Work Unit Number MF 58524001.0002.

J. D. MacEwen, Ph.D. served as the Laboratory Director for the THRU of the University of California, Irvine and as co-principal investigator with T. T. Crocker, M.D., Department of Community and Environmental Medicine. Acknowledgement is made to L. J. Jenkins, D. E. Uddin, C. D. Flemming and J. L. Monroe for their significant contributions and assistance in the conduct of the study and preparation of this report.

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INTRODUCTION

A 90-day continuous inhalation toxicity study of diesel fuel marine (DFM) vapor was conducted by the Toxic Hazards Research Unit (THRU). Samples of DFM derived from petroleum as well as oil shale sources were tested to determine if the two fuels presented different health hazards. Petroleum DFM has been used by the U. S. Navy as the fleet standard fuel for a variety of ships, while oil shale DFM has been developed as a possible replacement fuel.

As part of the overall evaluation of the oil shale fuels, it is desirable to assess the toxicity associated with typical use exposure. Data of this type allow for a comparison of the hazards of shale and petroleum derived fuels and are valuable in establishing proper workplace procedures and controls. Since inhalation will be the primary route of exposure for personnel working with DFM, inhalation exposures were conducted to compare the effects at potential exposure levels. A 90-day continuous inhalation exposure period was chosen to simulate conditions where Naval personnel may be exposed during a cruise situation. While this type of exposure is less traditional than a 6 hour/day, 5 day/week regimen, it does create a maximum exposure situation and increases the probability of observing exposure related effects.

DFM is typically a mixture of branched and cyclic hydrocarbons. The fuel contains a small amount of benzene which was considered to be a constituent of major toxicological interest. The reported effects of benzene exposure involve blood disorders with reductions in the number of erythrocytes, leukocytes, and platelets found in humans after long-term exposure to benzene at high concentrations (Greenberg et al., 1939; Hardy and Elkins, 1948; Aksoy et al., 1972).

A number of recent investigations with hydrocarbon fuels have shown that exposed male Fischer 344 rats often develop hyaline droplets and necrosis of the proximal tubular epithelium (Gaworski et al., 1985a; MacNaughton and Uddin, 1984; Parker et al., 1981). MacFarland et al. (1984) found similar kidney lesions in male rats exposed to unleaded gasoline. In addition, renal neoplasia developed in the male rats exposed for long periods to unleaded gasoline and several high density military fuels (Kitchen, 1984; Bruner, 1984).

METHODS

Test Material

DFM is described in military specification MIL-F-16884G (7 March 1973), from which the properties shown in Table 1 were selected. Petroleum and Shale DFM were supplied to the THRU by the Naval Medical Research Institute/Toxicology Detachment (NMRI/TD) in clean 55-gallon drums. The Petroleum DFM was obtained from stock supplies. The Shale DFM was refined from Paraho crude oil by the Sohio refinery in Toledo, Ohio, and designated by Sohio as FIN-DFM. The Shale DFM was shipped via rail tank car to the Naval Toxicology Detachment at Wright-Patterson Air Force Base where it was transferred to clean drums.

TABLE 1. MILITARY SPECIFICATIONS: DIESEL FUEL MARINE

Appearance:	Clear bright and free from visible particulate matter
Distillation Temperature (°C)	
Initial Boiling Point:	--
90% Recovery:	357°
End Point, Maximum Temperature:	385°
Sulfur, Total Weight, % Maximum:	1.00
Flashpoint, °C, Minimum:	60
Pour Point °C, Maximum:	-6.7
Viscosity at 100°F, Kinematic, Centistokes:	1.8-4.5

DFM Generation and Monitoring

The petroleum and oil shale studies were both conducted in a similar manner. The basic design for the DFM generation system was adapted from previous studies of hydrocarbon fuels. Since DFM is a multicomponent material with a wide boiling range, it was necessary to operate the animal exposure chambers from a single master generation system (Figure 1) to assure similar exposure. To reduce potential fire hazard, an overheat alarm with a fuel shut off capability was incorporated into the generation apparatus.

Two identically operated solvent evaporator towers were required to generate sufficient Petroleum DFM vapor for the assigned chamber concentrations. It was necessary to add a third vapor generating tower in order to produce enough vapor from the Shale DFM which was less volatile than previous hydrocarbon fuels studied.

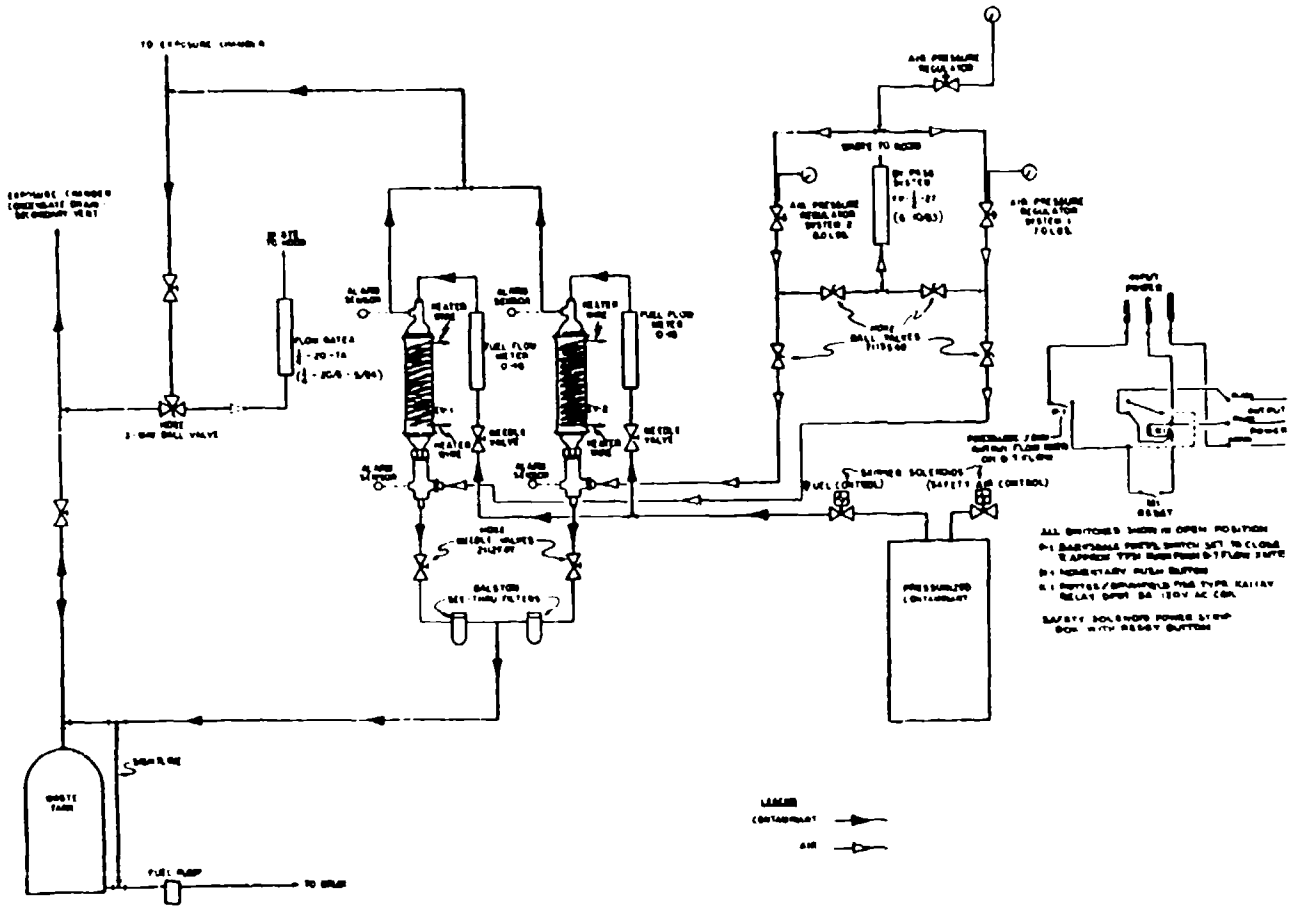

Figure 1. DFM contaminant introduction system.

Two of the evaporator tower outputs were mixed and split between the two exposure chambers. The output of the third tower went directly into the higher concentration chamber. Operating conditions of the evaporator towers were identically maintained to assure similarity in the DFM vapor constituents within the chambers. The central zone of the glass tower was a cylinder 13" long by 1-3/4" O.D. It had a 13-turn spiral 9" long impressed in the wall to hold a heating coil and lengthen the evaporation path. The top reduced to a "T" with a 1" O.D. right arm for vapor exhaust and a 1/4" O.D. connector for input fuel. The bottom reduced to a 1" O.D. glass connector. DFM was metered in the top of the electrically heated evaporator towers. Air entered the towers at the side near the bottom while spent fuel was drained from the bottom. The hot fuel vapor/air mix was

split in the approximate volume ratio of the chamber concentration and entered the respective chamber air streams. A double "T" of stainless steel tubing and pipe fittings allowed air input, spent fuel exit, and temperature monitoring probe on the waste fuel. A valve downstream controlled air/spent fuel flow to reduce vapor loss from the bottom of the tower. The primary source of heat was a 1/4" O.D. close coiled Nichrome wire (B & S-20 gauge, 1.1 Ω/inch, Wooge Manufacturing Company, Chicago, Illinois), approximately 72" in length. Tower temperature was maintained using a proportional temperature controller with a sensor monitoring output vapor temperature. Additional heat was added to the system by wrapping the metal fittings at the bottom of the tower with a heat tape, temperature controlled with a variable voltage transformer.

Rather large volumes (100-500 gallons) of fuel were necessarily present in the area adjoining the chambers for prolonged periods of time. Because the system had to operate unattended except for hourly operational checks during the study, an overheat alarm/shutdown system shown in Figure 2 was incorporated. Four temperature probes, each capable of system shutdown were placed at the most sensitive problem areas (fuel vapor/air mix leaving each tower and fuel draining from bottom of each tower). The temperature at each probe position was recorded hourly.

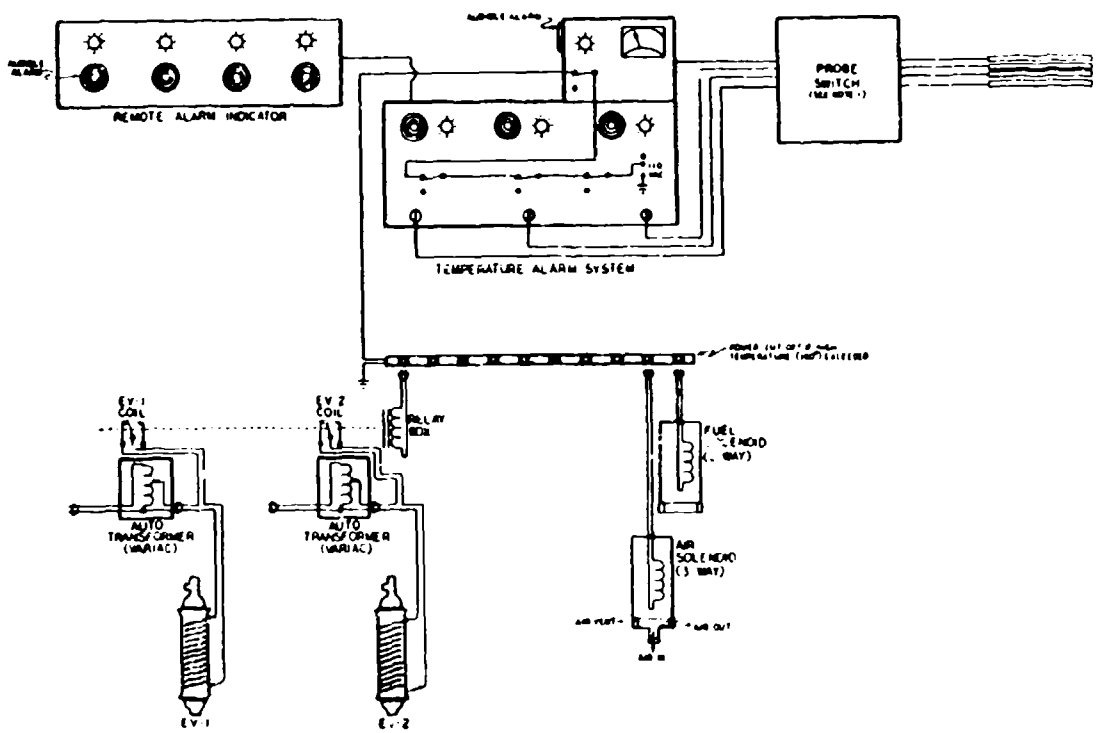

Figure 2. DPM contaminant introduction alarm system.

On emergency shutdown, all heater power was turned off, fuel flow stopped and fuel pressure relieved, and the fuel drums vented through the generation hood exhaust. In case of power failure, the whole system would shut down in the same manner. The temperature limit used was 120°F at the fuel vapor/air mix leaving the tower. Both upper and lower temperature sensor systems were set to trip off at 140°F.

The mass of hydrocarbon vaporized per unit time was dependent on the effective heat input while the vapor component concentration was affected by the ratio of components present in the fuel and the fuel flow rate. The system as operated for petroleum DFM had a fuel flow rate limit of about 15 mL/tower/minute, while 8 mL/tower/min was used in the shale DFM study.

A Beckman Model #400 hydrocarbon analyzer was used for mass analysis. Chamber concentrations were analyzed using a single analyzer by dilution of the higher DFM concentration chamber sample to a similar concentration as the low concentration using input chamber air for diluent and as the source of baseline air (Figure 3).

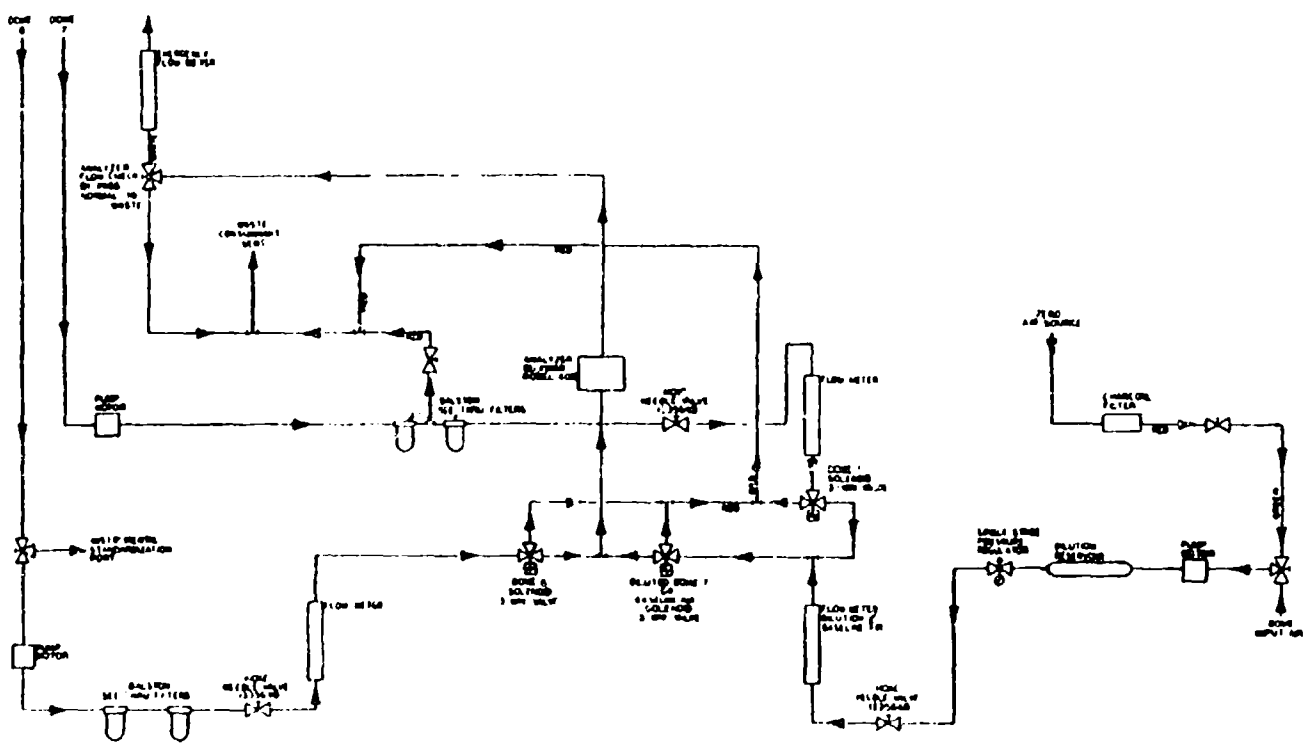

Figure 3. DFM sampling and analysis system.

Since the hydrocarbon analyzer response was directly related to the total carbon content of the sample, standardization was possible using a reliable defined system. Various concentrations of instrument grade propane (99^+ % as C_3), diluted in 100 L mylar bags, served as standards. Instrumental response was determined to be linear and stable for prolonged periods of time, provided the instrumental parameters were strictly maintained. Twenty-four hourly mean readings were used for daily concentration determinations.

In the petroleum DFM study, a Varian 1200 gas chromatograph (GC) equipped with a FID detector and a Spectra Physics Model I computing integrator was used for quality control checks of each drum of fuel prior to use, for spent fuel, and for generation system operation checks, and also chamber atmosphere fingerprint analysis. The GC was operated isothermally with the oven set at 40°C. Routine chromatographs were limited to peaks eluted in the first 20 minutes. The shale DFM study employed a Varian 3700 GC equipped with a FID detector and a Spectra Physics Model I computing integrator. This system allowed for a more complete fuel analysis using a temperature program function. Columns used for Petroleum or Shale DFM analysis were packed with either 10% SE 30 on Chromosorb W-AW or 10% SE 30 on Chromosorb W-HP.

A Royco 225 particle counter was used on a daily basis to monitor any condensate aerosol formed by components of the DFM generated into the chambers.

Animals

Young, adult purebred beagle dogs were selected from a colony maintained by the Air Force at Wright-Patterson Air Force Base. CDF (Fischer 344)/Crl/BR rats (9-11 weeks old) were purchased from Charles River Breeding Laboratories (Wilmington, Massachusetts). C57BL/6 mice (9-11 weeks old) were purchased from Jackson Laboratories (Bar Harbor, Maine). Test animals were gang-caged by species in stainless steel, wire-mesh cages during exposure. Animals had access to food (Purina, St. Louis, Missouri) and water ad libitum. All cage areas were cleaned daily during which time food remaining in the feeders was discarded and replaced with a fresh supply.

Exposure Conditions

Groups of 3 male and 3 female dogs, 150 male and 150 female rats, and 150 female mice were exposed via inhalation to 50 mg/m^3 or 300 mg/m^3 Petroleum or Shale DFM vapor continuously for a period of 90 days. Exposures were conducted in 25 m^3 inhalation chambers on a 24-hour basis and personnel servicing the chambers

during the exposure were provided with respiratory protection and disposable protective clothing. Control groups were maintained in Bioclean® laminar air flow rooms in a separate facility. The airflow, pressure, relative humidity, and temperature of each chamber were monitored and recorded hourly. Relative humidity was maintained at 50% ± 10 and the temperature at 22°C ± 2.

Upon termination of the 90-day exposure period, all of the dogs and 1/3 of the rodents from each group were killed for detection of pathologic lesions caused by exposure. The remaining rodents were held for long-term postexposure observation. An interim sacrifice was conducted at 19 months postexposure with a final sacrifice during the 24th month of the study.

All animals were carefully observed throughout the exposure and postexposure periods for signs of altered physical condition. Rats and dogs were weighed individually at bi-weekly intervals during exposure, and rats were weighed monthly during the postexposure period. Mice were weighed monthly throughout the study, and the group mean weights were monitored. All animals that died or were killed were necropsied. The following tissues were taken for histopathologic examination: adrenals, anus, urinary bladder, brain, colon, duodenum, esophagus, gallbladder, heart, ileum, kidneys, larynx, liver, lungs and bronchi, mammary gland, mesenteric and mandibular lymph nodes, nasal cavity, ovaries, pancreas, parathyroids, pituitary, prostate, salivary gland, sciatic nerve, seminal vesicles, skin, spleen, bone-sternebrae, vertebrae or femur (plus marrow), stomach, testes, thigh muscle, thymus, thyroids, trachea, and uterus. Dog red blood cell osmotic fragility tests were conducted at exposure termination. The liver, spleen, and kidneys of individual dogs and rats were weighed at exposure termination and, for surviving rats, at 19 months postexposure. At the conclusion of the Shale DFM study, kidney tissue from three male rats in each group was fixed by vascular perfusion with 2.5% glutaraldehyde and 2% paraformaldehyde buffered with 0.1 M cacodylate at pH 7.4. Thin sections were then prepared for Transmission Electron Microscopic examination. Blood samples were drawn from fasted dogs bi-weekly and from fasted rats at exposure termination and interim necropsy for hematology and clinical chemistry tests: hematocrit (HCT), hemoglobin (HGB), red blood cell (RBC) count, white blood cell (WBC) count, differentials, albumin, alkaline phosphatase, bilirubin, blood urea nitrogen (BUN), calcium, creatinine, glucose, potassium, glutamic-oxaloacetic transaminase (SGOT), glutamic-pyruvic transaminase (SGPT), sodium, and total protein.

Data Analysis

Body weights were analyzed by a repeated measures design using Scheffe pairwise comparisons. Blood test results and organ weights were analyzed by two factorial ANOVA with Scheffe pairwise comparisons. A linear trend and an overall chi-squared test were performed on the histopathologic lesions. If the lesion had a statistically significant overall chi-squared, pairwise comparisons were done using a Yates' corrected chi-squared or a Fisher exact test where appropriate (Zar 1974).

RESULTS

Exposure concentrations presented to the animals were well controlled throughout both studies. Table 2 shows the mean concentrations for the entire 90-day period.

TABLE 2. SUMMARY OF DFM EXPOSURE CONCENTRATION INFORMATION

	50 mg/m^3 Target Concentration	300 mg/m^3 Target Concentration
Petroleum DFM		
Mean (mg/m^3)	50	299
SD	1.2	4.6
Range (mg/m^3)	47-59	279-308
Shale DFM		
Mean (mg/m^3)	50	300
SD	0.5	3.0
Range (mg/m^3)	47-52	278-308

Benzene concentrations monitored during the Petroleum DFM study by gas chromatography demonstrated very low benzene concentrations at both exposure levels. At the 50 mg/m^3 target level the benzene concentration averaged about 0.13 mg/m^3 (0.04 ppm) and at the 300 mg/m^3 target level the benzene concentration averaged about 0.27 mg/m^3 (0.08 ppm). The use of the temperature programmed gas chromatographic technique used in the Shale DFM study indicated that the majority of the constituents in the liquid Shale DFM were between C_{12} and C_{19}, while the actual chamber atmosphere contained mostly C_8 to C_{12} fractions in measurable quantities (Table 3).

Analysis for condensate aerosol in the exposure chambers indicated negligible aerosol formation in either the Petroleum or Shale DFM study.

TABLE 3. GAS CHROMATOGRAPHIC ANALYSIS OF LIQUID SHALE DFM AND CHAMBER ATMOSPHERE[a]

	Liquid DFM		Chamber Atmosphere	
Fraction[b]	% of Total Area	Cumulative %	% of Total Area	Cumulative %
$<C_5$	N.I.[c]	-	≈ 1.00	≈ 1.00
C_5	N.I.	-	0.35	1.35
C_6	N.I.	-	0.83	2.18
C_7	N.I.	-	4.51	6.69
C_8	N.I.	-	12.59	19.28
C_9	0.90	0.90	16.41	35.69
C_{10}	0.98	1.88	18.60	54.29
C_{11}	1.81	3.69	21.71	76.00
C_{12}	4.22	7.91	14.08	90.08
C_{13}	10.90	18.81	6.93	97.01
C_{14}	15.56	34.37	0.44	97.45
C_{15}	16.77	51.15	0.63	98.08
C_{16}	14.89	66.03	N.I.	-
C_{17}	12.74	78.77	N.I.	-
C_{18}	13.33	92.10	N.I.	-
C_{19}	7.39	99.49	N.I.	-
C_{20}	0.52	100	N.I.	-

[a] The fractions were separated by a SE-30 chromosorb W-HP column. The area % was determined from the FID response using a Spectra Physics System I integrator.

[b] The fractions are designated by the normal alkane number and include all compounds between the previous normal alkane up to and including the designated normal alkane.

[c] Not integratable.

Dogs

Dogs exposed to either Petroleum or Shale DFM were generally heavier than unexposed controls (Table 4). Despite this weight difference, body weights were well within normal limits and the apparent effect was considered incidental. No mortalities occurred in dogs exposed to DFM.

Examination of the RBC osmotic fragility of dogs exposed to Petroleum DFM for 90 days indicated a non-dose related increase (Figure 4). Osmotic fragility curves for dogs exposed to Shale DFM for 90 days were not different from controls (Figure 4).

TABLE 4. EFFECT OF DFM EXPOSURE ON DOG BODY WEIGHT (kg)[a]

Time (wk)	Petroleum DFM Concentration (mg/m^3) 0	50	300
0	9.5 ± 0.6	10.1 ± 1.1	10.2 ± 0.6
2	9.8 ± 0.7	10.7 ± 1.0	10.7 ± 0.8
4	9.7 ± 0.7	10.5 ± 1.0	10.9 ± 1.0
6	9.6 ± 0.7	10.7 ± 1.1	11.2 ± 1.1[b]
8	9.9 ± 0.6	11.0 ± 1.1	11.3 ± 1.1[b]
10	9.8 ± 0.6	11.0 ± 1.0	11.8 ± 1.2[c]
12	10.3 ± 0.6	10.9 ± 1.1	11.7 ± 1.2[b]

Time (wk)	Shale DFM Concentration (mg/m^3) 0	50	300
0	9.6 [illegible]	10.9 ± 0.6[b]	9.9 ± 0.7
2	9.6 [illegible] .2	10.9 ± 0.5[b]	10.5 ± 0.7[b]
4	9.6 ± 0.3	11.4 ± 0.6[b]	10.9 ± 0.8[b]
6	10.4 ± 0.2	11.4 ± 0.6[b]	11.4 ± 0.7[b]
8	10.0 ± 0.2	11.7 ± 0.6[b]	11.9 ± 0.8[b]
10	10.5 ± 0.2	11.9 ± 0.7[b]	12.6 ± 0.7[b]
12	10.3 ± 0.2	12.1 ± 0.6[b]	12.3 ± 0.7[b]

[a] Mean ± SE, N = 6.
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.

The trend toward increased RBC fragility in dogs exposed to Petroleum DFM was not accompanied by any abnormal changes in other erythrocyte parameters that were periodically measured throughout the exposure. The only serum chemistry parameter that demonstrated any consistent trend occurred in dogs exposed to Shale DFM, where a slight but statistically significant increase in BUN was seen (Table 5). The elevated BUN in dogs exposed to the Shale DFM was clearly not dose related, however.

The absolute liver weight of dogs exposed to 300 mg/m^3 Petroleum DFM was significantly greater than controls (Table 6). However, the weight of this organ relative to body weight was comparable to the relative liver weight of the control group. Weights of other organs from dogs exposed to Petroleum DFM were normal. Dogs exposed to Shale DFM did not exhibit any organ weight changes that were considered exposure related.

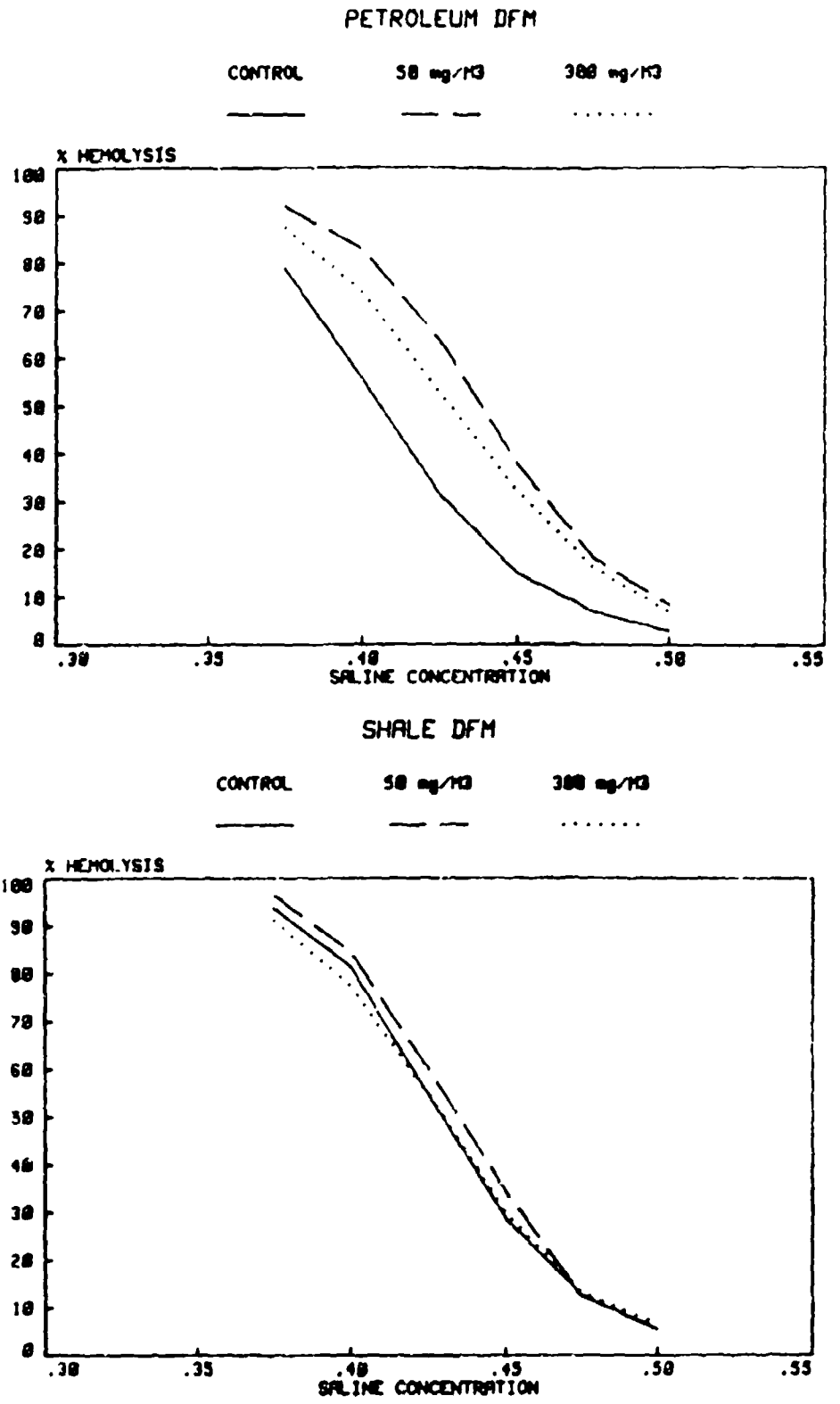

Figure 4. Effect of DFM exposure on dog red blood cell osmotic fragility.

TABLE 5. EFFECT OF DFM EXPOSURE ON DOG SERUM BUN LEVELS (IU/L)[a]

Time (wk)	Petroleum DFM Concentration			Shale DFM Concentration		
	0	50	300	0	50	300
0	14.3 ± 1.4	15.7 ± 1.0	16.1 ± 1.5	17.7 ± 2.0	14.2 ± 1.0[b]	15.0 ± 0.6
2	12.5 ± 1.0	19.5 ± 2.0[c]	16.6 ± 1.2[c]	11.8 ± 0.6	13.7 ± 0.8	14.8 ± 1.3
4	13.6 ± 1.0	14.0 ± 1.2	14.4 ± 1.0	10.5 ± 1.4	18.9 ± 1.9[c]	16.8 ± 1.1[c]
6	14.3 ± 1.1	14.9 ± 1.6	15.6 ± 0.8	12.0 ± 0.6	17.5 ± 2.0[c]	17.6 ± 1.0[c]
8	14.2 ± 0.8	15.6 ± 1.8	13.9 ± 0.4	12.2 ± 0.6	16.5 ± 0.9[c]	17.5 ± 1.1[c]
10	13.2 ± 1.1	16.3 ± 1.6[b]	16.6 ± 1.0[c]	15.7 ± 1.0	19.1 ± 2.0	20.6 ± 3.0[c]
12	14.3 ± 1.0	15.6 ± 2.8	15.6 ± 1.3	13.3 ± 0.4	20.3 ± 2.5[c]	17.9 ± 1.8[c]

[a] Mean ± SE, N = 5 or 6 samples/group.
[b] Different from control, $p < 0.05$.
[c] Different from control, $p < 0.01$.

TABLE 6. EFFECT OF DFM EXPOSURE ON DOG ORGAN WEIGHT[a]

	Exposure Termination					
	Petroleum Concentration (mg/m³)			Shale Concentration (mg/m³)		
	0	50	300	0	50	300
Body weight (kg)	10.3 ± 0.6	10.9 ± 1.1	11.7 ± 1.2	10.4 ± 0.3	11.9 ± 0.6[b]	12.0 ± 0.6[b]
Liver weight (g)	288.3 ± 18.5	329.3 ± 24.0	376.9 ± 24.2[c]	314.0 ± 12.5	327.1 ± 17.9	384.7 ± 20.3
Kidney weight (g)	54.4 ± 2.7	49.1 ± 4.9	53.2 ± 3.7	44.1 ± 0.8	51.2 ± 3.4	51.9 ± 4.1
Spleen weight (g)	78.0 ± 8.1	77.5 ± 11.8	60.9 ± 15.6	42.4 ± 7.1	93.5 ± 14.6[c]	86.3 ± 15.1
Liver % body	2.8 ± 0.1	3.1 ± 0.2	3.3 ± 0.2	3.0 ± 0.1	2.8 ± 0.1	3.2 ± 0.2
Kidney % body	0.5 ± 0.01	0.5 ± 0.1	0.5 ± 0.03	0.4 ± 0.01	0.4 ± 0.03	0.4 ± 0.02
Spleen % body	0.8 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.8 ± 0.1	0.7 ± 0.1

[a] Mean ± SE, N = 6 samples/group.
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.

Histopathologic examination of tissues harvested from dogs revealed that cytoplasmic vacuolization of hepatocytes occurred with slightly increased frequency in dogs exposed to Petroleum DFM (control - 2 of 6; 50 mg/m³ - 4 of 6, 300 mg/m³ - 5 of 6). This lesion was interpreted as a mild manifestation of cytotoxicity or a predisposition for hepatocytes to accumulate excessive glycogen. All other microscopic lesions noted in the dogs exposed to Petroleum DFM were considered incidental findings unrelated to exposure. No significant exposure or dose related lesions were observed in dogs exposed to Shale DFM.

Mice

Analysis of mouse survival indicated a reduced mean survival time in mice exposed to Shale DFM when compared to their respective control group (Table 7). It was noted that the majority of the deaths in the Shale DFM groups were moribund sacrifices due to ulcerative dermatitis. The overall incidence of this skin lesion was less in the mice used in the Petroleum

DFM study. This resulted in fewer moribund sacrifices. The mean survival times for Petroleum DFM exposed groups were comparable to the control group.

TABLE 7. SURVIVAL TIME OF MICE EXPOSED TO DFM

Treatment	Mean Survival Time (Month) ± SE	
	Petroleum DFM	Shale DFM
Control	20.9 ± 0.5	20.6 ± 0.4
50 mg/m^3	19.0 ± 0.6	17.6 ± 0.7[a]
300 mg/m^3	20.2 ± 0.5	16.9 ± 0.6[a]

[a] Significantly different, based on the Mantel-Cox test ($p = 0.0148$)

No body weight effects were seen in mice exposed to either Petroleum or Shale DFM.

Histopathologic findings in mice killed at the completion of the 90 day exposure included mild pulmonary inflammatory lesions in subjects assigned to the Shale DFM study (Table 8). Mice exposed to Petroleum DFM did not exhibit significant pulmonary inflammatory changes. Liver inflammatory changes consisting of multifocal accumulations of chronic inflammatory cells were noted in all groups (Table 8). Typically, these lesions were mild, involving only a few hepatocytes, and were not associated with a distinct etiologic factor or agent. Hepatocellular vacuolization/fatty change was noted more frequently in mice exposed to Shale DFM when compared to their control group. The distribution of the vacuolization was centrilobular in controls, but panlobular in Shale DFM exposed mice. The severity of the change was also considered to be greater in the exposed mice. Mice exposed to Petroleum DFM did not exhibit any increase in liver cell vacuolization or fatty change.

The vast majority of the pathologic lesions observed in mice held for the postexposure period were common changes attributable to aging processes. Most of these lesions were equally distributed among the exposed and non-exposed groups in both studies. Table 9 presents a list of the major changes noted in mice. Many of the common aging changes as well as lesions occurring with low incidence are not shown. There was a slightly increased incidence of ulcerative dermatitis in mice exposed to DFM.

TABLE 8. HISTOPATHOLOGIC LESIONS[a] IN MICE AT TERMINATION OF 90 DAYS OF CONTINUOUS INHALATION EXPOSURE TO DFM

	Petroleum DFM Concentration (mg/m³)			Shale DFM Concentration (mg/m³)		
	0	50	300	0	50	300
Lung						
Inflammatory Changes	5/46(11)	9/43(21)	0/42(0)	12/49(24)	34/49(69)[b]	38/48(79)[b]
Liver						
Inflammation	12/45(27)	15/43(35)	25/42(60)[b]	14/51(27)	23/49(47)[b]	9/48(19)
Vacuolization/ Fatty Change	12/45(27)	1/43(2)[b]	4/42(10)	18/51(35)	46/49(94)[b]	41/48(85)[b]

[a] Number observed/number examined (%).
[b] Different from control, $p < 0.01$.

TABLE 9. HISTOPATHOLOGIC LESIONS[a] IN MICE HELD FOR POSTEXPOSURE OBSERVATION AFTER 90-DAY CONTINUOUS INHALATION EXPOSURE TO DFM

	Petroleum DFM Concentration (mg/m³)			Shale DFM Concentration (mg/m³)		
Tissue	0	50	300	0	50	300
Skin						
Inflammation	15/87(17)	20/88(23)	24/91(26)	30/96(31)	41/97(42)	45/99(45)
Bone Marrow						
Hyperplasia	3/83(4)	4/87(5)	9/83(11)	17/90(19)	30/86(35)[b]	31/92(34)[b]
Fibrosis	3/83(4)	5/87(6)	6/83(7)	1/90(1)	0/86(0)	1/92(1)
Respiratory						
Nose -						
Hyaline degeneration	0/92(0)	0/87(0)	0/92(0)	23/96(24)	17/97(18)	13/98(13)
Crystals	1/92(1)	0/87(0)	0/92(0)	25/96(26)	19/97(19)	11/98(11)[c]
Lung -						
Crystals	19/91(21)	22/90(24)	13/94(14)	10/98(10)	11/94(12)	3/98(3)[c]
Alveolar adenoma	4/91(4)	4/90(4)	3/94(3)	5/98(5)	1/94(1)	4/98(4)
Alveolar carcinoma	0/91(0)	0/90(0)	2/90(2)	1/98(1)	1/94(1)	1/98(1)
Liver						
Vacuolization/ Fatty change	3/93(3)	2/91(2)	6/94(6)	14/97(14)	12/98(12)	16/97(16)
Adenoma	4/93(4)	6/91(7)	9/94(10)	1/97(1)	2/98(2)	0/97(0)
Carcinoma	1/93(1)	3/91(3)	0/94(0)	0/97(0)	0/98(0)	0/97(0)
Inflammation	0/93(0)	0/91(0)	0/94(0)	41/91(45)	17/98(17)[c]	19/97(20)[c]
Urinary						
Kidney -						
Hyaline degeneration	3/92(3)	1/91(1)	0/94(0)	10/94(11)	5/94(5)	2/97(2)[b]
Spleen						
Hematopoiesis	17/91(19)	28/89(31)	24/92(26)	24/95(25)	34/94(36)	38/96(40)
Endocrine						
Pituitary - adenoma	43/71(61)	38/77(49)	41/78(53)	25/81(31)	28/77(36)	14/76(18)
Thyroid -						
Papillary hyperplasia	40/82(49)	36/84(45)	40/82(49)	62/94(66)	51/92(55)	48/96(50)
Adenoma	9/82(11)	7/84(8)	4/82(5)	5/94(5)	2/92(2)	4/92(4)
Lymphoreticular						
Malignant lymphoma	37/93(40)	26/91(29)	29/94(31)	29/98(30)	29/99(29)	22/99(22)

[a] Number observed/number examined (%).
[b] Different from control, $p < 0.05$.
[c] Different from control, $p < 0.01$.

These skin lesions were generally regarded as common changes associated with fighting among cagemates. The role of DFM vapor in promoting increased dermal lesions in exposed mice was unclear. Secondary changes related to the dermatitis were bone marrow and splenic granulocytic hyperplasia (hematopoesis) and hepatocellular fatty change.

Hyaline degeneration of the respiratory epithelium, with subsequent crystal formation, is frequently observed in aging C57BL/6 mice used in this laboratory. Possible causes for this change have not been established; however, there has never been a distinct relationship with exposure to any test agent. Accordingly, the pathologist who examined petroleum DFM exposed mice did not document nasal hyaline degeneration in any subject whereas the pathologist assigned to the Shale DFM study did record this change as a possible lesion. Noteworthy is the fact that nasal hyaline degeneration and crystals were more common in control mice than in Shale DFM exposed animals. Most important with respect to respiratory tract lesions was the observation that primary lung neoplasms were not significantly increased in any group of DFM exposed mice. In some instances these entities observed in mice exposed to Shale DFM appeared to be related, in that crystals were observed arising from hyalinized respiratory epithelium. No significant lung tumors were noted in either study.

Liver cell vacuolization and fatty change, which had been increased in mice at termination of exposure to Shale DFM, was found with equal frequency in controls and exposed groups examined postexposure. In many cases, hepatocellular fatty change was believed to be secondary to the chronic, debilitating effects of ulcerative dermatitis which was common in all DFM study groups. Liver inflammation was increased at exposure termination in mice exposed to Petroleum DFM. This was not diagnosed in mice held for postexposure observation. The distribution of liver inflammation in mice from the Shale DFM study clearly demonstrates that DFM was not the causative agent. There was no significant increase in liver tumors in mice exposed to DFM.

No evidence of substantial kidney toxicity was observed. Renal tubular cell hyaline degeneration was not a significant finding. Endocrine system tumors and malignant lymphomas were common in all groups, with no relationship to DFM exposure.

Rats

Exposure to Petroleum or Shale DFM did not significantly alter survival time when compared to respective controls. All

groups from either study had mean survival times of approximately 22 months.

Body weights of male rats exposed to Petroleum or Shale DFM are shown in Figure 5. Exposure to Petroleum DFM resulted in a dose related decrease in weight gain. This effect occurred during the exposure phase and was present throughout most of the postexposure observation period. Shale DFM exposure at 300 mg/m^3 depressed male rat growth in a similar manner, with significant ($p < 0.01$) differences between this group and the control group evident during the entire study. Exposure to 50 mg/m^3 Shale DFM resulted in only transient weight differences from the control group.

Female rats exposed to Petroleum DFM demonstrated reduced body weight gain which became more pronounced during the post-exposure period (Figure 6). The effect was not dose related, however. Exposure to Shale DFM did not affect female rat body weight gain.

Male and female rat organ weights measured at exposure termination and 19 months postexposure are shown in Tables 10 and 11, respectively. Although there were statistically significant differences noted between DFM exposed groups and controls, no clear dose response relationship was apparent in any of the major organs measured. Spleens obtained from three female control rats in the Petroleum DFM study at 19 months postexposure were excessively large. Two of the spleens weighed in excess of 5 grams while the third weighed greater than 20 grams. WBC counts of these animals were in excess of 80,000 $cells/mm^3$. The findings were considered compatible with large granular lymphocytic leukemia. Spleen weights and blood values from these rats were excluded from statistical analysis.

Male rat hematology, BUN, and creatinine values measured during the study are shown in Table 12. At exposure termination male rats exposed to DFM demonstrated a trend toward reduced red blood counts, hematocrit, and hemoglobin levels. The decreases were generally small, but slightly more pronounced in rats exposed to the higher DFM concentration. This effect was not evident at the 19-month postexposure examination period. The BUN and creatinine levels of rats exposed to 300 mg/m^3 Shale DFM were elevated at exposure termination. This effect was not seen in rats exposed to Petroleum DFM. Increases in these two parameters, while being statistically significant, were not great enough to be considered substantial biological alterations. All other blood parameters examined in male rats were within normal limits, with no indication of any exposure related effects.

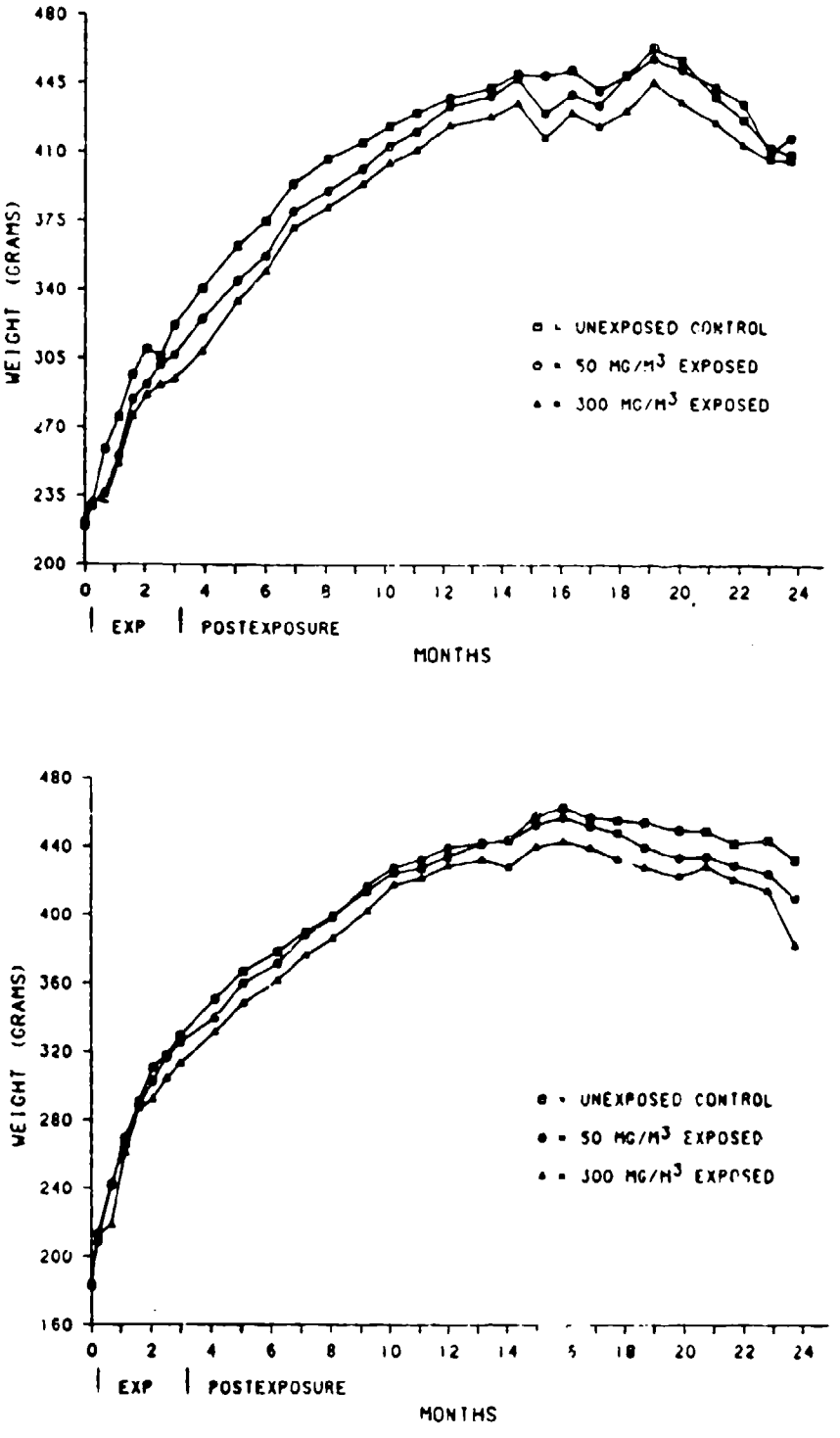

Figure 5. Effect of DFM exposure on male rat body weight. (Petroleum DFM top curve and Shale DFM bottom curve).

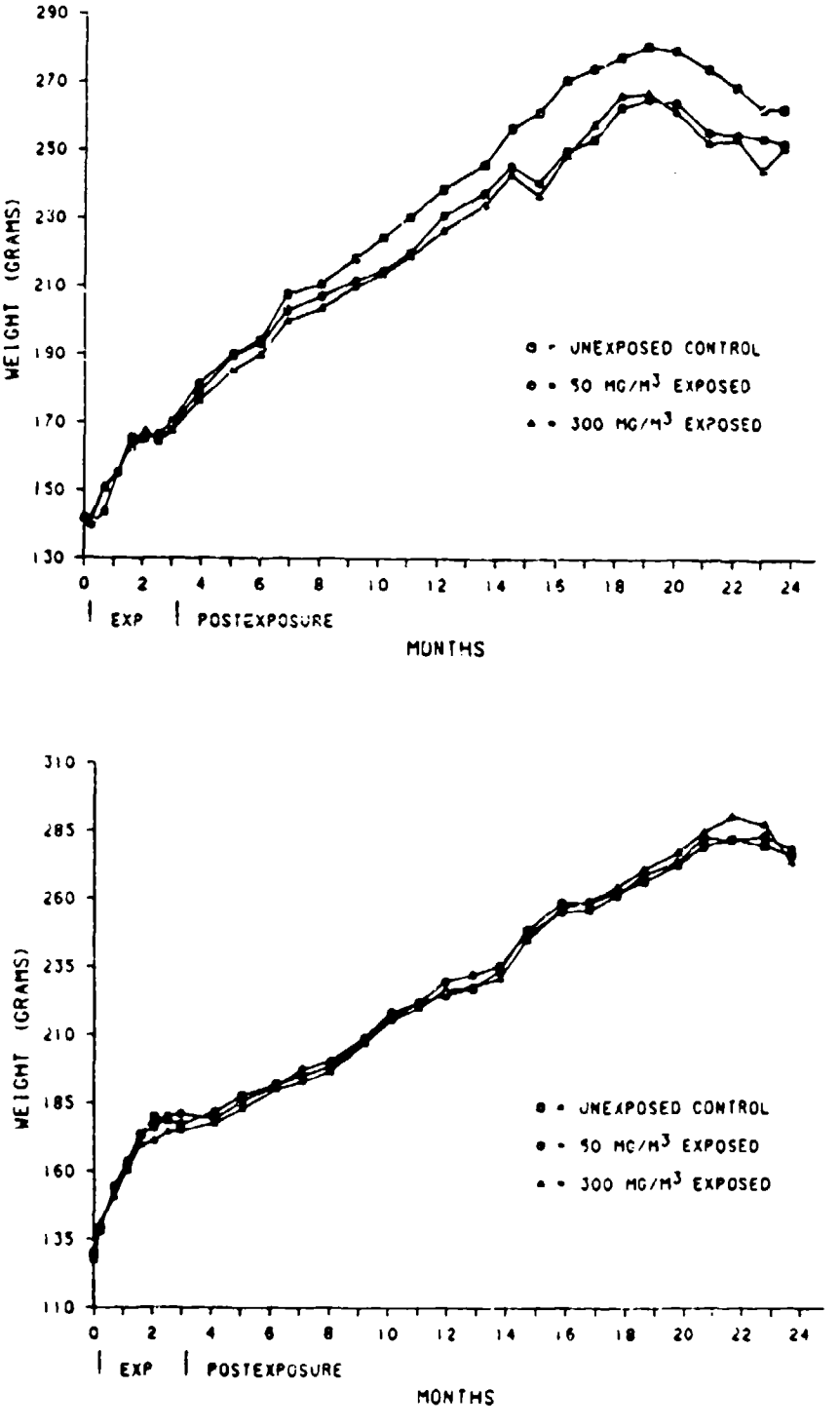

Figure 6. Effect of DFM exposure on female rat body weight. (Petroleum DFM top curve and Shale DFM bottom curve).

TABLE 10. EFFECT OF DFM EXPOSURE ON MALE RAT ORGAN WEIGHT[a]

	Exposure Termination					
	Petroleum DFM Concentration (mg/m³)			Shale DFM Concentration (mg/m³)		
	0	50	300	0	50	300
Body weight (g)	318 ± 3	303 ± 3[b]	302 ± 3[b]	310 ± 4	311 ± 4	306 ± 6[c]
Liver weight (g)	8.82 ± 0.11	8.43 ± 0.14[c]	8.78 ± 0.10	8.08 ± 0.15	7.76 ± 0.13	8.04 ± 0.18
Kidney weight (g)	2.13 ± 0.04	1.99 ± 0.05[c]	2.07 ± 0.03	2.07 ± 0.03	1.99 ± 0.03	2.24 ± 0.05[b]
Spleen weight (g)	0.48 ± 0.03	0.48 ± 0.02	0.51 ± 0.02	0.55 ± 0.01	0.57 ± 0.01	0.55 ± 0.01
Liver % body	2.77 ± 0.03	2.79 ± 0.04	2.91 ± 0.02[b]	2.60 ± 0.03	2.50 ± 0.02[c]	2.72 ± 0.03[b]
Kidney % body	0.67 ± 0.01	0.66 ± 0.01	0.69 ± 0.01	0.67 ± 0.01	0.64 ± 0.01[b]	0.76 ± 0.01[b]
Spleen % body	0.15 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.19 ± 0.01
	19 Months Posterposure					
	Petroleum DFM Concentration (mg/m³)			Shale DFM Concentration (mg/m³)		
	0	50[d]	300	0	50	300
Body weight (g)	420 ± 9	426 ± 9	404 ± 6	422 ± [illegible]	413 ± 7	399 ± 9
Liver weight (g)	12.46 ± 0.44	11.84 ± 0.31	11.31 ± 0.31[c]	12.81 ± 0.38	11.96 ± 0.33	12.12 ± 0.55
Kidney weight (g)	2.97 ± 0.06	2.86 ± 0.05	2.78 ± 0.06[c]	3.01 ± 0.07	2.87 ± 0.06	3.02 ± 0.11
Spleen weight (g)	0.99 ± 0.17	0.94 ± 0.13	0.99 ± 0.15	0.48 ± 0.08	0.53 ± 0.09	0.55 ± 0.08
Liver % body	3.01 ± 0.16	2.78 ± 0.06	2.82 ± 0.09	3.05 ± 0.10	2.91 ± 0.09	3.03 ± 0.10
Kidney % body	0.72 ± 0.03	0.67 ± 0.01	0.69 ± 0.02	0.72 ± 0.02	0.70 ± 0.01	0.76 ± 0.03
Spleen % body	0.24 ± 0.04	0.22 ± 0.03	0.25 ± 0.04	0.12 ± 0.02	0.13 ± 0.02	0.14 ± 0.02

[a] Mean ± SE. N = 18 to 25 samples/group.
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.
[d] N = 15.

TABLE 11. EFFECT OF DFM EXPOSURE ON FEMALE RAT ORGAN WEIGHT[a]

	Exposure Termination					
	Petroleum DFM Concentration (mg/m³)			Shale DFM Concentration (mg/m³)		
	0	50	300	0	50	300
Body weight (g)	169 ± 1	172 ± 1	172 ± 1	169 ± 2	168 ± 2	165 ± 2
Liver weight (g)	4.78 ± 0.08	4.07 ± 0.06[b]	4.78 ± 0.10	4.24 ± 0.07	4.11 ± 0.10	4.43 ± 0.08
Kidney weight (g)	1.35 ± 0.01	1.13 ± 0.01[b]	1.23 ± 0.02[b]	1.20 ± 0.02	1.12 ± 0.02[b]	1.16 ± 0.02
Spleen weight (g)	0.37 ± 0.01	0.37 ± 0.01	0.34 ± 0.02	0.37 ± 0.01	0.37 ± 0.01	0.37 ± 0.01
Liver % body	2.84 ± 0.04	2.37 ± 0.03[b]	2.78 ± 0.05	2.51 ± 0.02	2.44 ± 0.04	2.68 ± 0.04[b]
Kidney % body	0.80 ± 0.01	0.66 ± 0.01[b]	0.71 ± 0.01[b]	0.71 ± 0.01	0.66 ± 0.01[b]	0.70 ± 0.01
Spleen % body	0.22 ± 0.01	0.22 ± 0.01	0.20 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.23 ± 0.01
	19 Months Posterposure					
	Petroleum DFM Concentration (mg/m³)			Shale DFM Concentration (mg/m³)		
	0	50	300	0	50	300
Body weight (g)	2[illegible] ± 5	247 ± 5	246 ± 6	265 ± 7	263 ± 7	278 ± 6
Liver weight (g)	7.64 ± 0.22	6.98 ± 0.16[c]	6.76 ± 0.19[b]	6.83 ± 0.09	6.85 ± 0.29	6.99 ± 0.16
Kidney weight (g)	2.17 ± 0.13	1.95 ± 0.05	1.83 ± 0.04[c]	1.87 ± 0.03	1.86 ± 0.05	1.87 ± 0.04
Spleen weight (g)	0.52 ± 0.03	0.45 ± 0.02	0.65 ± 0.15	0.50 ± 0.02	0.52 ± 0.03	0.55 ± 0.03
Liver % body	2.97 ± 0.11	2.82 ± 0.04	2.75 ± 0.04	2.62 ± 0.08	2.62 ± 0.09	2.52 ± 0.03
Kidney % body	0.84 ± 0.06	0.79 ± 0.02	0.75 ± 0.02	0.72 ± 0.03	0.72 ± 0.03	0.67 ± 0.01
Spleen % body	0.20 ± 0.01	0.18 ± 0.01	0.26 ± 0.06	0.19 ± 0.01	0.20 ± 0.01	0.20 ± 0.01

[a] Mean ± SE, N = 18 to 25 samples/group.
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.

TABLE 12. EFFECT OF DFM EXPOSURE ON MALE RAT BLOOD[a]

	Exposure Termination					
	Petroleum DFM Concentration (mg/m³)			Shale DFM Concentration (mg/m³)		
	0	50	300	0	50	300
RBC (x10⁶ cells/mm³)	8.43 ± 0.10	8.17 ± 0.11	7.71 ± 0.11[b]	8.93 ± 0.09	8.51 ± 0.12[b]	8.30 ± 0.09[b]
WBC (x10³ cells/mm³)	7.8 ± 0.2	5.6 ± 0.2[b]	5.2 ± 0.2[b]	5.6 ± 0.2	5.9 ± 0.2[c]	5.9 ± 0.2[b]
HCT (%)	48 ± 0.3	47 ± 0.4[c]	44 ± 0.4[b]	45 ± 0.2	45 ± 0.3	43 ± 0.2
HGB (gm/dL)	15.5 ± 0.1	15.3 ± 0.1	14.7 ± 0.1[b]	15.1 ± 0.2	15.2 ± 0.2	14.8 ± 0.2
BUN (mg/dL)	16.9 ± 0.5	17.4 ± 0.5	16.7 ± 0.4	16.5 ± 0.4	16.4 ± 0.3	17.9 ± 0.4[c]
Creatinine (mg/dL)	0.7 ± 0.02	0.7 ± 0.02	0.7 ± 0.02	0.4 ± 0.02	0.5 ± 0.02[b]	0.6 ± 0.02[b]
	19 Months Postexposure					
	Petroleum DFM Concentration (mg/m³)			Shale DFM Concentration (mg/m³)		
	0	50	300	0	50	300
RBC (x10⁶ cells/mm³)	7.93 ± 0.27	8.92 ± 0.28[c]	8.94 ± 0.27[b]	8.01 ± 0.31	7.36 ± 0.47	7.39 ± 0.28
WBC (x10³ cells/mm³)	6.2 ± 0.4	6.8 ± 0.4	6.1 ± 0.4	6.8 ± 0.9	6.6 ± 0.8	5.7 ± 0.3
HCT (%)	46 ± 2	50 ± 2	50 ± 2	49 ± 2	47 ± 3	48 ± 1
HGB (gm/dL)	15.1 ± 0.7	16.2 ± 0.6	16.5 ± 0.6	16.9 ± 0.6	15.4 ± 0.8	15.9 ± 0.4
BUN (mg/dL)	19.3 ± 3.5[d]	14.2 ± 0.6[e]	15.8 ± 0.5[f]	18.6 ± 0.9	17.9 ± 1.2	22.9 ± 5.3
Creatinine (mg/dL)	0.9 ± 0.1[d]	0.7 ± 0.03[ce]	0.8 ± 0.04[c]	0.6 ± 0.03	0.6 ± 0.03	0.8 ± 0.2

[a] Mean ± SE, 15 to 25 samples/group.
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.
[d] N = 8.
[e] N = 9.
[f] N = 13.

Hematology, BUN, and creatinine values of female rats are shown in Table 13. Female rats exposed to Petroleum DFM had slightly reduced erythrocyte parameters in comparison with controls; however, the effect was not dose related. Shale DFM exposure did not alter female rat hematological values. All other blood measurements in female rats during the study were within normal species variation and any differences between exposed and control rats were considered to be unrelated to exposure.

Histopathologic examination of rats killed immediately following the 90-day exposure revealed slight increases in acute and chronic inflammatory lesions in the nasal mucosa of male and female rats exposed to Petroleum DFM (Table 14). Lymphoid hyperplasia of the bronchial submucosa occurred in most groups in the petroleum study, and was slightly more prevalent in petroleum DFM exposed females when compared with controls. It should be emphasized that peribronchial lymphoid hyperplasia is regarded as a common aging change in F-344 rats and often is not documented consistently by histopathologists. No significant lesions were noted in the respiratory tract of rats killed immediately following exposure to Shale DFM.

TABLE 13. EFFECT OF DFM EXPOSURE ON FEMALE RAT BLOOD[a]

	Exposure Termination					
	Petroleum DFM Concentration (mg/m³)			Shale DFM Concentration (mg/m³)		
	0	50	300	0	50	300
RBC (x10⁶ cells/mm³)	7.23 ± 0.10	6.78 ± 0.08[b]	6.90 ± 0.11[c]	7.84 ± 0.13	8.02 ± 0.09	7.86 ± 0.08
WBC (x10³ cells/mm³)	7.7 ± 0.5	6.8 ± 0.5	5.3 ± 0.3[b]	4.2 ± 0.2	4.7 ± 0.3	3.9 ± 0.2
HCT (%)	43 ± 0.2	41 ± 0.4[b]	42 ± 0.4	42 ± 0.2	42 ± 0.3	41 ± 0.2
HGB (gm/dL)	14.5 ± 0.1	14.1 ± 0.1[c]	14.2 ± 0.1[c]	14.6 ± 0.1	14.9 ± 0.1	14.6 ± 0.1
BUN (mg/dL)	16.9 ± 0.6	17.0 ± 0.7	17.4 ± 0.6	16.3 ± 0.6	17.4 ± 0.6	17.4 ± 0.7
Creatinine (mg/dL)	0.6 ± 0.02	0.5 ± 0.02[c]	0.6 ± 0.03	0.4 ± 0.02	0.5 ± 0.02[c]	0.4 ± 0.03
	19 Months Postexposure					
	Petroleum DFM Concentration (mg/m³)			Shale DFM Concentration (mg/m³)		
	0	50	300	0	50	300
RBC (x10⁶ cells/mm³)	8.88 ± 0.13[d]	8.84 ± 0.11	8.36 ± 0.28	8.13 ± 0.15	7.61 ± 0.17[c]	8.22 ± 0.28
WBC (x10³ cells/mm³)	5.1 ± 0.3	5.3 ± 0.4	5.0 ± 0.4	3.5 ± 0.1	4.1 ± 0.3	3.7 ± 0.2
HCT (%)	46 ± 0.4[d]	45 ± 0.5	43 ± 1[c]	46 ± 0.3	44 ± 1	45 ± 0.5
HGB (gm/dL)	15.2 ± 0.2[d]	15.2 ± 0.2	14.4 ± 0.5	15.3 ± 0.1	15.1 ± 0.2	15.4 ± 0.2
BUN (mg/dL)	15.2 ± 0.8[e]	15.7 ± 0.7[f]	16.0 ± 0.5	12.9 ± 0.3	12.1 ± 0.5	12.6 ± 0.5
Creatinine (mg/dL)	0.7 ± 0.1[e]	0.7 ± 0.1[f]	0.7 ± 0.03	0.4 ± 0.02	0.4 ± 0.02	0.5 ± 0.03

[a] Mean ± SE, 18 to 25 samples/group.
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.
[d] N = 15.
[e] N = 4.
[f] N = 11.

TABLE 14. HISTOPATHOLOGIC LESIONS[a] IN RATS OBSERVED AT TERMINATION OF 90 DAYS OF CONTINUOUS INHALATION EXPOSURE TO DFM

	Male Rats					
	Petroleum DFM Concentration (mg/m³)			Shale DFM Concentration (mg/m³)		
	0	50	300	0	50	300
Respiratory						
Nasal inflammation	0/25(0)	1/25(4)	3/25(12)	5/25(20)	1/24(4)	0/23(0)[b]
Lung - Bronchial lymphoid hyperplasia	12/25(48)	16/25(64)	13/25(52)	0/25(0)	1/23(4)	0/25(0)
Kidney						
Hyaline degeneration	0/25(0)	17/25(68)[c]	21/25(84)[c]	0/26(0)	23/24(96)[c]	23/25(92)[c]
Necrosis	0/15(0)	0/25(0)	24/25(96)[c]	0/25(0)	0/24(0)	25/25(100)[c]
Interstitial tissue inflammation	6/25(24)	1/25(4)	0/25(0)[b]	4/25(16)	4/24(17)	21/25(84)[c]
	Female Rats					
	Petroleum DFM Concentration (mg/m³)			Shale DFM Concentration (mg/m³)		
	0	50	300	0	50	300
Respiratory						
Nasal inflammation	0/25(0)	8/25(32)[c]	7/25(28)[c]	5/22(23)	0/25(0)[b]	1/24(4)
Lung - Bronchial lymphoid hyperplasia	7/24(28)	15/25(60)	14/25(56)	0/25(0)	0/25(0)	0/25(0)
Kidney						
Hyaline degeneration	0/25(0)	0/25(0)	0/25(0)	0/21(0)	0/25(0)	0/24(0)
Necrosis	0/25(0)	0/25(0)	0/25(0)	0/21(0)	0/25(0)	0/24(0)
Interstitial tissue inflammation	1/25(4)	0/25(0)	0/25(0)	3/21(14)	0/25(0)	1/24(4)

[a] Number observed/number examined (%).
[b] Different from control, $p < 0.05$.
[c] Different from control, $p < 0.01$.

At exposure termination, the most striking lesions seen in rats exposed to DFM occurred in the kidneys of males (Table 14). Renal tubular hyaline droplet degeneration was observed in most of the male rats exposed to either Petroleum or Shale DFM for 90 days. Necrosis of the renal tubular epithelial cells was evident in multifocal regions of the outer and middle cortex in virtually all of the male rats exposed to 300 mg/m^3 DFM, and it was considered to be a consequence of the hyaline degenerative process. Necrosis was not a distinctive feature of the kidneys of male rats exposed to 50 mg/m^3 DFM. Associated with the necrosis of the tubular epithelium was the presence of multiple, moderately dilated renal tubules near the corticomedullary junction which contained prominent plugs of eosinophilic granular debris, compatible with casts of desquamated tubular epithelial cells. Mild, chronic inflammation of the renal cortical interstitial tissue was also observed with increased frequency in the male rats exposed to Shale DFM at 300 mg/m^3, and was often associated with early degenerative tubular lesions. This inflammatory process was characterized by focal to multifocal aggregations of lymphocytes, plasma cells, macrophages, and was occasionally accompanied by the early deposition of fibrous connective tissue.

Transmission electron microscopic examination of the kidney of male rats in the Shale DFM study revealed distinct cytotoxic alterations in the proximal tubular epithelial cells attached to the basal lamina. These cells contained numerous variably sized, angulated, membrane-bound, osmophilic granules in the cytoplasm. Granules were observed at all levels in the cell. In some instances they appeared as dark crystalline inclusions surrounded by less dense granular matrix in structures thought to be lysosomes. Others showed a slight gradation of electron densities suggesting coalescence of smaller granules to form larger ones. Microvilli were absent on the luminal surfaces of some degenerating cells while others demonstrated club-like swelling of microvillar tips. Additionally, prominent vacuoles and/or spaces were present extending from the luminal to the basilar surfaces of many tubular cells. Many of these vacuolated areas were interpreted as dilated lateral intercellular spaces between adjacent cells. However, some spaces contained degenerating, crystalline material and subcellular organelles. Several tubules had denuded regions where epithelial cells had exfoliated, exposing the underlying peritubular basal lamina. Exfoliated cells were often observed as intraluminal debris at many levels of the proximal tubule.

Ultrastructural lesions observed in the medulla were characterized by microcystic tubular dilatation at the junction of the

outer and inner stripe of the outer medulla where the straight segment of the proximal tubule narrows to become the descending loop of Henle. Cells lining the cysts were ultrastructurally compatible with those of the proximal descending limb. They were moderately compressed by casts of necrotic debris within the lumen, but were thought to be viable.

Results of microscopic examination of the tissues collected from male rats maintained for postexposure observation are shown in Table 15. The list has been abbreviated by excluding the lesions that occurred with low frequency and were considered incidental to exposure. The clearest indication of an exposure related effect was again demonstrated in the kidneys of male rats. Virtually all of the male rats, controls included, developed lesions characteristic of chronic progressive renal nephropathy (CPN); however, CPN lesions were more severe in the male rats exposed to 300 mg/m^3 DFM when compared to the other groups in each respective study. The most striking difference between DFM exposed rats and unexposed controls was the development of tubular mineralization and pelvic epithelial hyperplasia. Furthermore, a clear dose response relationship was demonstrated for these changes. Virtually all of the male rats exposed to 300 mg/m^3 had mineralized deposits in the renal papilla. The incidence of mineralization in male rats exposed to 50 mg/m^3 Shale DFM was about 50%, while the incidence of this change in male rats exposed to 50 mg/m^3 Petroleum DFM was less than the background level in the control group. The mineralized deposits were considered to be calcium impregnated necrotic cell debris shed from the tubule during the exposure phase. In addition to the mineralization, a dose-dependent, papillary hyperplasia of the transitional epithelium was present over the renal papillus. Etiologic factors for this urothelial hyperplasia were obscure, but were attributed to the mechanical irritation of the mineralized debris or diminished compliance of the kidney secondary to CPN.

Two benign kidney tumors were seen in the studies. One developed in a male rat exposed to 50 mg/m^3 Petroleum DFM, while the other was observed in a male rat exposed to 300 mg/m^3 Shale DFM (Table 15). No kidney tumors were seen in control male rats. Tumors and tissue changes occurred in several other organ systems of male rats. In general, however, the frequencies of occurrence were equally distributed among the exposed and control groups for the respective studies. Furthermore, the lesions noted were consistent with changes normally seen in aged rats.

TABLE 15. HISTOPATHOLOGIC LESIONS[a] IN MALE RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 90 DAYS CONTINUOUS INHALATION EXPOSURE TO DFM

Tissue	Petroleum DFM Concentration (mg/m³) 0	50	300	Shale DFM Concentration (mg/m³) 0	50	300
Skin						
Mammary gland -						
Hyperplasia/dilatation	6/36(17)	1/35(3)	0/35(0)[b]	12/42(29)	7/44(16)	16/45(36)
Fibroadenoma	1/36(3)	0/35(0)	0/35(0)	0/42(0)	3/44(7)	4/45(9)
Cardiovascular						
Myocardial fibrosis/ degeneration	32/50(64)	23/49(47)	32/50(64)	46/49(94)	40/48(83)	45/51(88)
Pulmonary artery mineralization	5/50(10)	6/50(12)	9/50(18)	26/49(53)	16/48(33)	22/51(43)
Respiratory						
Nose - inflammation	4/50(8)	2/48(4)	4/49(8)	12/50(24)	16/47(34)	17/49(35)
Lung -						
Inflammation	23/50(46)	23/50(46)	20/50(40)	3/50(6)	21/49(43)[b]	14/50(28)[b]
Alveolar adenoma	1/50(2)	2/50(4)	1/50(2)	0/50(0)	0/49(0)	1/50(2)
Alveolar carcinoma	1/50(2)	0/50(0)	1/50(2)	0/50(0)	0/49(0)	1/50(2)
Liver						
Focal cell change	28/50(56)	20/50(40)	30/50(60)	30/49(60)	30/50(60)	25/50(50)
Bile duct hyperplasia	44/49(90)	44/50(88)	46/50(92)	49/49(100)	46/50(92)	46/50(92)
Carcinoma	0/50(0)	1/50(2)	2/50(4)	1/49(2)	1/50(2)	0/50(0)
Adenoma	2/50(4)	1/50(2)	0/50(0)	3/49(6)	0/50(0)	0/50(0)
Urinary						
Kidney -						
Nephropathy	50/50(100)	48/49(98)	49/50(98)	50/50(100)	49/49(100)	49/49(100)
Papillary hyperplasia	7/50(14)	2/49(4)	15/50(30)[b]	4/50(8)	15/49(30)[b]	42/49(86)[b]
Mineralization	5/50(10)	2/49(4)	42/50(84)[b]	5/50(10)	26/49(52)[b]	49/49(100)[b]
Adenoma	0/50(0)	1/49(2)	0/50(0)	0/50(0)	0/49(0)	1/49(2)
Reproductive & Endocrine						
Pituitary -						
Adenoma	18/48(38)	13/45(29)	14/47(30)	14/48(29)	8/47(17)	8/45(18)
Carcinoma	0/48(0)	0/45(0)	0/47(0)	0/48(0)	1/47(2)	3/45(7)
Thyroid -						
Follicular cell tumors	0/47(0)	0/46(0)	0/44(0)	4/50(8)	5/47(11)	0/50(0)
C cell tumors	1/47(2)	3/46(7)	3/46(7)	0/50(0)	0/47(0)	0/50(0)
Hyperplasia	5/47(11)	2/46(4)	8/46(17)	24/50(48)	18/47(38)	16/50(32)
Parathyroid -						
Hyperplasia	1/40(3)	1/40(3)	1/38(3)	1/43(2)	1/33(3)	5/34(15)
Adenoma	0/40(0)	0/40(0)	1/38(3)	1/43(2)	1/33(3)	1/34(3)
Testes -						
Interstitial cell tumor	45/49(92)	40/47(85)	46/50(92)	45/49(92)	44/47(94)	46/47(98)
Prostate -						
Inflammation	3/44(7)	2/39(5)	1/40(3)	10/42(24)	8/43(14)	14/45(31)
Hyperplasia	2/44(5)	2/39(5)	2/40(5)	10/42(24)	10/43(23)	14/45(31)
Adrenal -						
Focal cell change	1/50(2)	0/50(0)	0/49(0)	2/50(4)	5/50(10)	10/50(20)[c]
Cortical adenoma	1/50(2)	1/50(2)	2/49(4)	0/50(0)	1/50(2)	0/50(0)
Pheochromocytoma	4/50(8)	3/50(6)	6/49(24)	5/50(10)	10/50(20)	4/50(8)
Lymphoreticular						
Mononuclear cell leukemia	3/50(6)	4/50(8)	7/50(14)	7/50(14)	8/50(16)	3/50(6)

[a] Number observed/Number examined (%).
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.

Lesions identified in female rats maintained for postexposure observation are shown in Table 16. Female rats from all groups, including controls, developed mild renal lesions consistent with CPN. There was no indication of an exposure related increase in severity as was seen with male rats. Female rats in the Shale DFM study demonstrated mineralization of the renal papilla. The severity of this lesion was characterized as mild, with no indication of a dose related increase in frequency of occurrance. Papillary hyperplasia was not a significant finding in female rats from either study. No kidney tumors developed in female rats exposed to either Petroleum or Shale DFM, while one kidney tumor was found in a female control rat in the Petroleum DFM study. Other lesions noted in female rats exposed to DFM were considered to be normal aging processes. No other organ systems in female rats developed signs of significant DFM related tissue changes.

DISCUSSION

The development of male rat nephrotoxicity in rats exposed to DFM is consistent with the effects reported with a number of other hydrocarbon fuels and solvents (Carpenter et al., 1975a, 1975b; Gaworski et al., 1985a, 1985b; Parker et al., 1981; Phillips and Egan, 1984a, 1984b). Nephrotoxic changes consisted primarily of proximal tubular hyaline droplet degeneration with subsequent tubular cell necrosis noted in male rats exposed to 300 mg/m^3. Transmission electron micrographs obtained during the Shale DFM study showed large osmophilic granules that correlated well with the hyaline droplets detected by light microscopy. Most of the granules were thought to be secondary lysosomes formed after fusion of primary lysosomes with endocytotic vacuoles containing electron dense material. The electron micrographs implicate the proximal tubule as the primary location of the hydrocarbon-associated cytotoxic effect on the nephron. There was distinct cytolysis of the tubular epithelium in that nephron segment. It appeared that only a relatively few epithelial cells undergoing degeneration terminated in necrosis. The pattern of necrosis was unique inasmuch as some of the ultrastructural architecture of the cell seemed to be retained. It seemed that complete enzymatic degradation was somehow inhibited. The mechanism of precisely how that effect occurred remains undetermined. This electron microscopic study supported the light microscopic evidence that the medullary cysts in the region of the proximal descending limb of the loop of Henle resulted from cellular debris which had exfoliated from more

proximal segments of the nephron. Changes occurring in the kidney subsequent to exposure were mineralization of cell debris, urothelial papillary hyperplasia, and more severe chronic progressive nephropathy.

TABLE 16. HISTOPATHOLOGIC LESIONS[a] IN FEMALE RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 90 DAYS CONTINUOUS INHALATION EXPOSURE TO DFM

Tissue	Petroleum DFM Concentration (mg/m³) 0	50	300	Shale DFM Concentration (mg/m³) 0	50	300
Skin						
Mammary gland -						
Hyperplasia/dilitation	36/45(8)	34/43(79)	36/43(84)	18/50(36)	14/45(3)	15/46(33)
Adenocarcinoma	1/45(2)	0/43(0)	0/43(0)	1/50(2)	0/45(0)	0/46(0)
Fibroadenoma	3/45(7)	2/43(5)	8/43(19)	3/50(6)	1/45(2)	4/46(9)
Cardiovascular						
Myocardial fibrosis/ degeneration	13/49(27)	8/50(16)	9/49(18)	26/50(52)	31/50(62)	18/50(36)[b]
Pulmonary artery mineralization	3/50(6)	7/50(14)	2/49(4)	11/50(22)	16/50(32)	10/50(20)
Respiratory						
Nose - inflammation	3/50(6)	2/50(4)	2/49(4)	1/50(2)	0/50(0)	3/50(6)
Lung -						
Inflammation	28/49(57)	16/50(32)[b]	17/49(35)	3/50(6)	10/50(20)[b]	4/50(8)
Adenoma	1/49(2)	0/50(0)	0/49(0)	0/50(0)	0/50(0)	0/50(0)
Liver						
Focal cell change	14/50(28)	23/49(47)	22/49(44)	25/50(50)	22/50(44)	18/50(36)
Bile duct hyperplasia	14/50(28)	12/49(24)	12/50(24)	39/50(78)	42/50(84)	38/50(76)
Adenoma	1/50(2)	0/49(0)	1/50(2)	1/50(2)	0/50(0)	0/50(0)
Urinary						
Kidney -						
Nephropathy	38/48(79)	29/50(58)[b]	29/48(60)[b]	29/48(60)	31/46(67)	26/49(53)
Papillary hyperplasia	0/48(0)	0/50(0)	1/48(2)	1/48(2)	1/46(2)	0/49(0)
Mineralization	9/48(19)	3/50(6)	2/48(60)[b]	21/48(44)	20/46(43)	14/49(29)
Adenoma	1/48(2)	0/50(0)	0/48(0)	0/48(0)	0/46(0)	0/49(0)
Reproductive & Endocrine						
Pituitary -						
Adenoma	29/49(50)	24/45(9)	25/48(52)	16/49(32)	14/46(30)	16/46(35)
Carcinoma	0/49(0)	0/45(0)	2/48(4)	0/49(0)	1/46(2)	1/46(2)
Thyroid -						
Follicular cell tumors	0/41(0)	0/48(0)	1/46(2)	1/50(2)	3/49(6)	3/46(7)
Hyperplasia	3/41(7)	3/48(7)	2/46(4)	19/50(38)	21/49(42)	20/46(43)
Parathyroid - adenoma	1/34(3)	0/33(0)	0/36(0)	0/38(0)	0/35(0)	1/33(3)
Adrenal -						
Cell change	2/50(4)	5/50(10)	4/49(8)	9/50(18)	9/49(18)	13/49(27)
Carcinoma	1/50(2)	0/50(0)	0/49(0)	0/50(0)	0/49(0)	0/49(0)
Adenoma	0/50(0)	0/50(0)	1/49(2)	1/50(2)	0/49(0)	1/49(2)
Pheochromocytoma	2/50(2)	1/50(2)	3/49(6)	0/50(0)	3/49(6)	2/49(4)
Uterus -						
Carcinoma	2/46(4)	1/48(2)	5/49(10)	0/49(0)	0/49(0)	1/50(2)
Stromal polyps	8/46(4)	14/48(29)	11/49(22)	1/49(2)	1/49(2)	2/50(4)
Lymphoreticular						
Mononuclear cell leukemia	8/50(16)	5/50(10)	8/49(16)	3/50(6)	3/50(6)	4/50(8)

[a] Number observed/Number examined (%).
[b] Different from control, $p < 0.05$.

The results of microscopic examination of kidney tissue suggest that the lower dose utilized in this study may have approached a "no effect" level for the development of hydrocarbon associated renal nephropathy. In a previous 90-day continuous exposure to JP-5, the lowest concentration tested, 150 mg/m^3, produced structural alterations in male rat kidneys including necrosis at exposure termination with mineralization and papillary hyperplasia developing postexposure (Gaworski et al., 1985a). In the present study exposure to 50 mg/m^3 produced hyaline degenerative changes without evidence of tubular cell necrosis at exposure termination. Subsequent postexposure examination indicated no substantial mineraliztion nor papillary hyperplasia in male rats exposed to 50 mg/m^3 Petroleum DFM. Although these changes were seen in male rats exposed to Shale DFM at 50 mg/m^3, the incidence was considerably less than that occurring in male rats exposed to a higher concentration.

Although there were kidney tissue changes in male rats at the microscopic level, there was no indication of increased kidney weight, nor was there any suggestion of substantial functional changes, indicated by serum BUN and creatinine elevations. Male rats exposed to either Petroleum or Shale DFM had decreased weight gains compared to their respective controls. The relationship of this weight effect to the development of renal toxicity is unknown. Erythrocyte parameters of male rats exposed to DFM tended to show slight reductions when compared to controls. This effect was consistent with the trends noted in a previous 90-day continuous exposure to JP-5 jet fuel (Gaworski et al., 1985a), although DFM exposure failed to reduce RBC counts, hematocrit, and hemoglobin levels in all cases.

The absence of renal toxicity in female rats, female mice, and male and female dogs exposed to DFM is consistent with the theory that the development of hydrocarbon-induced nephrotoxicity is related to the presence of a protein unique to male rats. Alden et al. (1984) has identified alpha 2u globulin as the protein responsible for the formation and accumulation of hyaline droplets. Probably causative mechanisms include the combination of hydrocarbons (or metabolites) with alpha 2u globulin to form a complex which is indigestible by tubular cell lysosomal enzymes.

In a recently completed study of the effects of inhaled gasoline vapors, increased renal neoplasia was reported (MacFarland et al., 1984; Kitchen, 1984). Similar findings have been presented by Bruner (1984) concerning several high-density hydrocarbon missile fuels. In the present study of DFM, kidney tumors were identified in one male rat exposed to Petroleum DFM

at 50 mg/m^3 and in one male rat exposed to Shale DFM at 300 mg/m^3. No control male rats from either study developed renal tumors; however, a female control rat in the petroleum study did develop a renal neoplasm. The development of tumors in the two male rats exposed to DFM is most probably an incidental occurrence, although kidney tumors in rats are generally regarded as rare. Since the present study utilized a relatively short term 13-week continuous exposure compared to the greater than 100-week intermittent exposure conducted in the unleaded gasoline study, the possible development of kidney tumors in male rats exposed to DFM for longer time periods should not, however, be totally disregarded.

In general, the effects noted in the other species exposed to DFM were very mild. Changes noted in dog blood parameters, including red blood cell osmotic fragility, body weights, organ weights, as well as microscopic examination of tissues did not establish any clear exposure related effects. Mice exposed to Shale DFM developed lung inflammation and fatty change in the liver, while mice exposed to Petroleum DFM were generally free of these changes. Fatty change in the liver is often regarded as a non-specific tissue alteration. The lung and liver changes in Shale exposed mice were reversible as indicated by the absence of any significant increase in these lesions postexposure. The solvent nature of DFM was probably responsible for the increased occurrence of dermatitis in exposed mice, which ultimately was responsible for the slightly decreased length of survival noted in the Shale DFM exposed mice. The findings in this study suggest that both DFM fuels are closely comparable in toxicologic and carcinogenic properties.

In conclusion, the results of this study are in close agreement with the effects noted in other studies of hydrocarbon fuels. Male rat nephrotoxic changes were evident in animals exposed to either Petroleum or Shale DFM at 300 mg/m^3. A dose response was suggested by the reduced severity of nephrotoxicity in male rats exposed to 50 mg/m^3 Shale DFM. Furthermore, the absence of significant renal changes in male rats exposed to Petroleum DFM at 50 mg/m^3 indicates that this concentration may be near a "no effect" level for male rat renal toxicity.

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